## II. REMARKS

Upon entry of the present amendment, claims 1 to 7, and 57 to 71 will be pending.

### A. Regarding the Amendments

Claim 8 is cancelled herein without disclaimer, and without prejudice to Applicants pursuing prosecution of subject matter encompassed within the claim in an application claiming the benefit of priority of the subject application.

Claim 1 has been amended to delete reference to a reporter polypeptide being an enzyme (but see new claims 59 to 66). In addition, claim 1 has been amended to clarify that the "activity" of the reporter polypeptide is "transcriptional activity".

New claims 59 to 66 have been added. New claim 59 is based on previously pending claim 1, except that new claim 59 recites only to a reporter polypeptide being "an enzyme" (compare claim 1, directed to transcriptional activator reporter polypeptide). New claims 60 to 63 are based on previously pending claims 2 to 4 and 7, respectively. New claim 64 is based on previously pending claim 58. New claim 65 is based on previously pending claim 8 (now cancelled). New claim 66 is supported, for example, at page 10, lines 8-10.

New claims 67 to 71 also have been added. New claim 67 is based on previously pending claims 1 and 57, directed to a transcription factor reporter polypeptide. New claims 68 and 69 are based on previously pending claims 2 and 3, respectively. New claim 70 is based on previously pending claims 4 and 7. New claim 71 is based on previously pending claim 6. As such, new claims 59 to 71 are supported by the specification, and by the claims as previously pending, which are supported for reasons of record.

### B. Claim Objections

It is stated in the Office Action that "Claim 6 is objected to because of the following informalities: Claims 5, 6 and 8 are objected to because they depend from rejected claims 3 and 1. Appropriate correction is required." (Office Action, page 2).

Applicants are uncertain as to the basis of the objection, but believe the Examiner may be indicating that claim 6, which is objected to, would otherwise be allowable if amended to recite the limitations of claims 5, 3 and 1, from which claim 6 depends. If correct, then it would appear that reference to "claim 8" in the objection may be an error.

Although Applicants acknowledge the allowability of claim 6, it is submitted, for the reasons set forth below, that claims 1, 3 and 5, from which claim 6, also are allowable. As such, claim 6 has not been amended to an independent form (but see new claims 67 to 71). If the basis of the objection has been misunderstood, clarification is respectfully requested.

**C. Rejections under 35 U.S.C. § 112**

The objection to the specification and corresponding rejection of claims 1 to 4, 7 and 58 under 35 U.S.C. § 112, first paragraph, as allegedly lacking an adequate written description are respectfully traversed.

It is stated in the Office Action that there is no description of even a single species of a fusion protein, wherein the reporter polypeptide is an enzyme, the activity of which is repressed by conferring specific localization in a cell. While it is acknowledged that the specification discloses examples of enzyme reporter polypeptides and of repressor polypeptides, it is alleged that such disclosure does not adequately support those fusion proteins comprising an enzyme reporter polypeptide because there is no predictability of structure for the claimed genus and no exemplified species.

It is noted that the rejection is relevant only to new claims 59 to 66, which are directed to a fusion protein, which comprises an enzyme reporter polypeptide linked to a linker polypeptide comprising a protease cleavage site, and a repressor polypeptide that represses the enzymatic activity of the reporter polypeptide by conferring specific localization in a cell of the fusion protein, wherein, upon cleavage at the protease cleavage site, an increase in enzyme activity can be detected.

It is submitted that the skilled artisan, viewing the subject application, would have known Applicants were in possession of the claimed fusion proteins comprising an enzyme reporter polypeptide. More specifically, the specification discloses that a repressor polypeptide can be a signal peptide, which directs secretion of a fusion protein of the invention into an extracellular space or external environment (page 14, lines 16-20), or can be, for example, a nuclear localization sequence or mitochondrial localization signal (page 14, lines 25-27). It is submitted that it is well known that enzymes have specific requirements for activity, including, for example, pH, ionic strength, co-factor requirements, and access to an appropriate substrate. As such, the skilled artisan, viewing the subject application, would have known, for example, that a kinase reporter polypeptide that is linked to a signal peptide, would be exported from a cell and, therefore, would not have access to an intracellular substrate, thus exhibiting reduced activity. Further, even if such a kinase fusion protein had access to an appropriate substrate, it would be unlikely that appropriate conditions for kinase activity would be present in the extracellular environment, for an enzyme that otherwise is only active intracellularly. Further, the artisan would have known that, upon cleavage at the protease site in the linker of such a fusion protein, the kinase would no longer be exported from a cell and, therefore, would demonstrate increased enzymatic activity. Similarly, the artisan would have known that a fusion protein comprising a kinase, which phosphorylates a substrate in the cytoplasm, that is linked, for example, to a mitochondrial localization signal (repressor polypeptide), would be transported into mitochondria, thus demonstrating reduced activity, and that such activity would be increased upon cleavage at the protease site due to release of the kinase from the repressor polypeptide.

For the above reasons, it is submitted that one skilled in the art, viewing the specification, would have known that Applicants were in possession of fusion proteins comprising an enzyme reporter polypeptide, as claimed. Accordingly, it is respectfully requested that the objection to the specification be withdrawn, and that the corresponding rejection of the claims as lacking an adequate written description be removed.

The objection to the specification and corresponding rejections of claims 1 to 4, 7 and 58 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement are respectfully traversed.

It is alleged that specification does not disclose how to make a fusion protein comprising an enzyme reporter polypeptide, the activity of which is repressed by conferring specific localization in a cell. It is noted that the rejection is relevant only to the subject matter of new claims 59 to 66.

Applicants submit that methods of making fusion proteins, including methods of making fusion proteins that comprise a heterologous cell localization domain, were well known in the art at the time the subject application was filed. As such, undue experimentation would not have been required to make a fusion protein comprising, for example, a kinase linked to a signal peptide such that the kinase would be exported from a cell in which it otherwise has activity. Further, it is submitted that the skilled artisan reasonably would have known that the enzyme reporter polypeptide of such a fusion protein would have reduced activity outside of a cell due, for example, to lack of access to an intracellular substrate, and would have known that the enzyme reporter, upon cleavage from the signal peptide, would remain in the cell and, thereby, exhibit increased activity. For these reasons, it is submitted that the specification enables the fusion proteins encompassed within claims 59 to 66 such that one skilled in the art would have known how to make and use the claimed compositions without undue experimentation. Accordingly, it is respectfully requested that the objection to the specification be withdrawn, and that the corresponding rejections of the claims as allegedly lacking enablement be removed.

**D. Prior Art Rejections**

The rejection of claims 1 to 3, 5, 57 and 58 under 35 U.S.C. § 102(b) as allegedly anticipated by Sakai et al. is respectfully traversed.

It is stated in the Office Action that Sakai et al. describe a H-Ras/SREBP-2 fusion protein comprising an NH2-terminal sequence that projects into the cytosol and comprises a

transcription factor ("reporter polypeptide"), linked to a membrane anchor ("repressor polypeptide"), and that the fusion protein further comprises a protease cleavage site near a transmembrane domain that, when cleaved, releases the NH2-terminal segment, which enters the nucleus and activates transcription. By way of clarification, Applicants point out that the NH2-terminal segment of the fusion protein of Sakai et al. consists of the H-Ras sequence (see Figure 3A, page 1040), and that H-Ras is not a transcription factor *per se*, though it can activate transcription via a signal transduction pathway; H-Ras is a GTP binding protein (see page 1039, right column, first full paragraph). Further, Sakai et al. report that the H-Ras segment, upon cleavage, localized to the cytosol (paragraph bridging pages 1039-1040), not to the nucleus. Also, Sakai et al. do not appear to report whether transcriptional activity was increased in the test cells, but only show the results of immunoblot analyses, which reveal various forms of the chimeric protein (precursor, intermediate, or mature form; see, e.g., Figures 3B and 3C) as visualized using an antibody to an epitope tag (page 1039, right column, second full paragraph).

Since H-Ras is not a transcription factor, Sakai et al. would not appear to be relevant to the subject matter of claims 5, 6, and 67 to 71. However, because H-Ras can be a transcriptional activator and has GTPase (enzymatic) activity, it can be considered a "reporter polypeptide" encompassed within the subject matter of claims 1 to 4, 7, and 58 to 64.

It is submitted that Sakai et al. do not teach or suggest a fusion protein of the invention because the SREBP-2 portion of the fusion protein of Sakai et al. contains two protease cleavage sites (see Figure 3A), including a first cleavage site, which, upon cleavage, generates an H-Ras/SREBP-2 intermediate that remains membrane bound, and a second cleavage site, which, when cleaved, releases the H-Ras component to the cytosol (see Figures 3B and 3C; see, also, page 1039, right column, second full paragraph, and paragraph bridging pages 1039-1040). As shown in the model presented in Figure 1 of Sakai et al., cleavage at the first cleavage is not sufficient to release the H-Ras component from the cell membrane. Further, the second cleavage cannot occur absent the first cleavage (see page 1038, paragraph bridging columns). Thus, the fusion protein of Sakai et al. contains two protease cleavage sites, both of which must be cleaved

in a specified order for release of the H-Ras component from a cell membrane (see, also, Title of Sakai et al. reference). As such, Sakai et al. do not teach or suggest a fusion protein containing a protease cleavage site, which, when cleaved results in increased activity of a reporter polypeptide and, therefore, do not anticipate the claimed fusion proteins. As such, it is submitted that the reference does not anticipate the claimed invention and, therefore, requested that the rejection of the claims as lacking novelty over Sakai et al. be removed.

Further, as indicated above, H-Ras is not a transcription factor, and Sakai et al. do not teach or suggest a fusion protein comprising a transcription factor. As such, the reference clearly does not anticipate the subject matter of claims 5, 6 and 67 to 71. Accordingly, it is respectfully requested that the rejection of the claim 5 as lacking novelty over Sakai et al. be removed, and that the rejection is not relevant to the subject matter of claims 67 to 71.

The rejection of claims 1 to 3, 5, 57 and 58 under 35 U.S.C. § 102(e) as allegedly anticipated by Crabtree et al. is respectfully traversed.

It is stated that Crabtree et al. describe chimeric proteins comprising at least one ligand binding domain fused to an additional "action" domain, which can be a transcriptional activator, and that the chimeric protein can further contain an intracellular targeting domain. Applicants point out, however, that the Crabtree et al. generally teach chimeric proteins that oligomerize, including, for example, as homodimers or heterodimers, due to the linking action of bivalent ligands (see, e.g., column 3, lines 21-33, and lines 58-53; column 9, lines 46-57; and Figure 14). Notably, it appears that the chimeric proteins of Crabtree et al. are active only upon oligomerization (see Abstract). As such, when considered in its entirety, the Crabtree et al. reference does not appear to be particularly relevant to the claimed compositions.

Further, Applicants point out that the chimeric proteins of Crabtree et al. are readily distinguishable from the claimed fusion proteins. For example, the fusion proteins of Crabtree et al. are designed to localize to a cellular compartment in which they have activity (see, e.g., column 13, lines 43-45, describing a chimeric protein comprising a transcription factor linked to

a nuclear localization domain). In contrast, the claimed fusion proteins have reduced activity due to the specific localization in a cell conferred by the repressor domain. In addition, Crabtree et al. do not teach or suggest linking an "action" domain and a "localization" domain with a linker polypeptide containing a protease cleavage site, and do not appear to consider cleavage of their fusion proteins as a means to increase activity. As such, Crabtree et al. do not teach or suggest the claimed fusion proteins and, therefore, cannot anticipate the claimed invention. Accordingly, it is respectfully requested that the rejection of the claims as lacking novelty over Crabtree et al. be removed.

In view of the amendments and the above remarks, it is submitted that the claims are in condition for allowance, and a notice to that effect is respectfully requested. The Examiner is invited to contact Applicant's undersigned representative if there are any questions relating to the subject application.

Please charge any additional fees, or make any credits, to Deposit Account No. 50-1355.

Respectfully submitted,

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Richard J. Imbra
Registration No. 37,643
Telephone: (858) 677-1496
Facsimile: (858) 677-1465

**USPTO CUSTOMER NUMBER 28213**
GRAY CARY WARE & FREIDENRICH LLP
4365 Executive Drive, Suite 1100
San Diego, California 92121-2133

Enclosure: Petition for Extension of Time